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Salient findings on characterization and diversity analysis of *Rhizoctonia bataticola* causing emerging dry root rot of chickpea in Karnataka

Gururaj Sunkad, Deepa Dore, Meghana Patil, Ranjana Joshi and Mediga Kasi Rao

Abstract
Dry root rot is becoming an emerging endemic and economically important soil borne disease of chickpea in Karnataka. *Rhizoctonia bataticola*, is a soil borne fungus resulting in significant yield losses. Hence, the present investigation was carried out to understand the pathogen characters and diversity of its isolates under changing climatic scenario. Sixty isolates of *R. bataticola* were isolated from disease samples collected from different geographic regions of Karnataka and subjected to cultural and morphological diversity analysis using standard methodologies. Later, twenty representative isolates were studied for molecular characterization using universal ITS primers and sequencing was done for eight isolates. Finally, genetic diversity analysis was carried out by RAPD primers. All sixty isolates showed variation with respect to cultural and morphological characters. Universal ITS primers produced amplified product size of 550- 600 bp in all the twenty isolates indicating that isolates belong *to Rhizoctonia bataticola*. Further, nucleotide equencing was done for ITS region of 18S rRNA in eight isolates pathogen selected from eight geographic regions. Four random RAPD primers generated reproducible polymorphism and amplified products with all the primers showed polymorphic and distinguishable banding pattern indicating the genetic diversity among twenty representative isolates of *R. bataticola*.

Keywords: Chickpea, diversity, *R. bataticola*, dry root rot, management

Introduction
Chickpea (*Cicer arietinum* L.) is the world's second most important pulse crop after common bean, it is self-pollinated, diploid, annual and cool season legume crop and grown in around 50 countries under rainfed conditions. It is originated in South-Eastern Turkey and the adjoining Northern region of Syria. Globally, the annual production of chickpeas was 14.25 million metric tonnes in 2019, of which India alone contributes to nearly 60% of the total production (FAOSTAT, 2019). In the major chickpea-growing belts of India, dry root rot caused by *Rhizoctonia bataticola* (Taub.) Butle is becoming an economically important disease and results in huge losses wherever chickpea is grown. The farmers are facing the problem of disease in all states that grow the crop and it is particularly more in Karnataka. The recent reports indicated that dry root rot is an emerging as a potential threat to chickpea productivity and production (Ghosh *et al.*, 2013) [3]. The pathogen, is a soil and seed borne necrotrophic fungal pathogen that has a global distribution. Diversity is the genetic property of an organism to change its characters from one generation to the other. The disease has become a major threat to chickpea production in recent years due to changing climatic scenario and loss caused by the disease ranged from 5-80 % (Rai *et al*., 2022) [8]. Higher temperature and soil moisture depletion during crop growth period particularly at pre-harvesting stage is predisposing chickpea to dry root rot (Sharma and Pande, 2013) [3]. *Rhizoctonia bataticola* is a major threat to chickpea production in tropics and subtropics. The information on accurate detection and diversity of pathogen with respect to cultural and morphological characters as well as at molecular level is scanty. Hence, the present study focused on characterization of pathogen based on cultural and morphological characters as well as at molecular level using molecular techniques.

Material and Methods

Isolation and maintenance of the pathogen

Chickpea plants showing typical symptoms of the disease were collected from farmers fields growing chickpea in different geographic regions of Karnataka. Sixty pathogen isolates were obtained by standard tissue isolation on Potato Dextrose Agar (PDA) medium, later purified by hyphal tip technique.

Cultural diversity in *R. bataticola:* Cultural characters such as radial growth, colony morphology, colony pigmentation and aerial mycelium were studied by growing *R. bataticola* isolates on PDA by inoculating seven-day old cultures. The observations were recorded seventh day after inoculation in all sixty isolates.

Morphological diversity *R. bataticola:* Morphological characters such as width of the hyphae, micro-sclerotial production, number of days to produce microsclerotia as well as number of microsclerotia per microscopic field and shape were recorded by using seven-day old culture in all sixty isolates. To record shape and size, Q-capture image analyzer was used.

Molecular characterization of *R. bataticola* isolates: Twenty isolates of *R. bataticola* representing different geographic regions were selected and used for detection using ITS-1 (5′CCTGTGCACCTGTGAGACAG-3′) and ITS-4 (5′-TGTCCAAGTCAATGGACTAT-3′) forward and reverse primers by subjecting to PCR.

Genetic diversity in *R. bataticola* isolates: Genetic diversity in twenty isolates of *R. bataticola* was carried out by isolation of DNA and RAPD analysis according to Liu *et al*. (2000) and Rohlf (1998) using four random RAPD primers, OPC-06 (GAACGGACTC), OPG-15 (ACTGGGACTC), OPP-14 (CCAGCCGAAC) and OPU-07 (CCTGCTCATC). Later, genetic dissimilarity estimates for twenty isolates were employed to generate dendrogram. Based on simple matching co-efficient, a genetic similarity matrix was constructed to assess the genetic relatedness among the isolates of *R. bataticola* based on RAPD analysis.

Table 1: Radial growth of *R. bataticola* isolates on potato dextrose agar medium

Sl. No.	Isolate	Mycelial growth rate (mm)	Number of isolates
1	Rb- 1, Rb- 2, Rb- 3, Rb- 4, Rb- 5, Rb- 6, Rb- 7, Rb- 8, Rb- 10, Rb- 11, Rb- 12, Rb- 13, Rb- 14, Rb- 15, Rb- 16, Rb- 17, Rb- 18, Rb- 19, Rb- 20, Rb- 21, Rb- 22, Rb- 23, Rb- 24, Rb- 25, Rb- 26, Rb- 27, Rb- 28, Rb- 29, Rb- 30, Rb- 31, Rb- 32, Rb- 33, Rb- 34, Rb- 35, Rb- 36, Rb- 37, Rb- 38, Rb- 39, Rb- 40, Rb- 42, Rb- 43, Rb- 44, Rb- 45, Rb- 46, Rb- 47, Rb- 48, Rb- 49, Rb- 50, Rb- 51, Rb- 52, Rb- 53, Rb- 54, Rb- 56, Rb- 57, Rb- 59	Fast (75-90 mm)	55
2	Rb- 55, Rb- 58	Moderate (60-75 mm)	2
3	Rb- 9, Rb- 41, Rb- 60	Slow (45-60 mm)	3

Table 2: Colony morphology of *R. bataticola* isolates on potato dextrose agar medium

Sl. No.	Isolate	Colony morphology	Number of isolates
1	Rb- 5, Rb- 8, Rb- 10, Rb- 11, Rb- 23, Rb- 26, Rb- 27, Rb- 34, Rb- 38, Rb- 39, Rb- 41, Rb- 48, Rb- 49, Rb- 50, Rb- 51, Rb- 54, Rb- 53	Appressed	17
2	Rb- 1, Rb- 2, Rb- 3, Rb- 4, Rb- 9, Rb- 17, Rb- 18, Rb- 28, Rb- 43, Rb- 46, Rb- 47, Rb- 55, Rb- 52	Velvety	13
3	Rb- 6, Rb- 7, Rb- 12, Rb- 13, Rb- 14, Rb- 15, Rb- 16, Rb-19, Rb- 20, Rb- 21, Rb- 22, Rb- 24, Rb- 25, Rb- 29, Rb- 30, Rb- 31, Rb- 32, Rb- 33, Rb- 35, Rb- 36, Rb- 37, Rb- 40, Rb- 42, Rb- 44, Rb- 45, Rb- 56, Rb- 57, Rb- 58, Rb- 59, Rb- 60	Fluffy	30

Table 3: Colony pigmentation of isolates of *R. bataticola* on potato dextrose agar medium

Sl. No.	Isolate	Colony pigmentation	Number of isolates
1	Rb- 1, Rb- 2, Rb- 3, Rb- 20, Rb- 28, Rb- 30, Rb- 31, Rb- 32, Rb- 42, Rb- 43, Rb- 45, Rb- 47, Rb- 49, Rb- 56, Rb- 57	Black with grey aerial mycelium	15
2	Rb- 12, Rb- 13, Rb- 15, Rb- 18, Rb- 19, Rb- 25, Rb- 26, Rb- 27, Rb- 29, Rb- 34, Rb- 35, Rb- 39, Rb- 48, Rb- 51, Rb- 60	Black	15
3	Rb- 4, Rb- 7, Rb- 8, Rb- 9, Rb- 10, Rb- 11, Rb- 14, Rb- 16, Rb- 17, Rb- 21, Rb- 23, Rb- 24, Rb- 33, Rb- 36, Rb- 37, Rb- 38, Rb- 40, Rb- 41, Rb- 44, Rb- 46, Rb- 50, Rb- 52, Rb- 53, Rb- 59, Rb- 58	Grey	25
4	Rb- 5, Rb- 6, Rb- 22, Rb- 55, Rb- 54	White pista	5

Table 4: Aerial mycelium production in isolates of *R. bataticola* on potato dextrose agar medium

Sl. No.	Isolate	Aerial mycelia	Number of isolates
1	Rb- 27, Rb- 28, Rb- 29, Rb- 30, Rb- 24, Rb- 25, Rb- 21, Rb- 22, Rb- 19, Rb- 20, Rb- 15, Rb- 16, Rb- 6, Rb- 1, Rb- 31, Rb- 32, Rb-33, Rb- 36, Rb- 37, Rb- 40, Rb- 43, Rb- 44, Rb- 45, Rb- 46, Rb- 47, Rb- 49, Rb- 56, Rb- 57	Present	28
2	Rb- 2, Rb- 3, Rb- 4, Rb- 5, Rb- 7, Rb- 8, Rb- 9, Rb- 10, Rb- 11, Rb- 12, Rb- 13, Rb- 14, Rb- 17, Rb- 18, Rb- 23, Rb- 26, Rb- 27, Rb- 34, Rb- 35, Rb- 38, Rb- 39, Rb- 41, Rb- 48, Rb- 50, Rb- 51, Rb- 52, Rb- 53, Rb- 54, Rb- 55, Rb- 58, Rb- 59, Rb- 60	Absent	32

Table 5: Microsclerotial production in isolates of *R. bataticola* on potato dextrose agar

Sl. No.	Isolate	Microsclerotia	Number of isolates
1	Rb-1, Rb-2, Rb-3, Rb-4, Rb-7, Rb-8, Rb-9, Rb-10, Rb-11, Rb-12, Rb-13, Rb-14, Rb-15, Rb-17, Rb-18, Rb-19, Rb-20, Rb-23, Rb-25, Rb-26, Rb-27, Rb-28, Rb-29, Rb-30, Rb-31, Rb-32, Rb-33, Rb-34, Rb-35, Rb-36, Rb-37, Rb-38, Rb-39, Rb-40, Rb-41, Rb-42, Rb-44, Rb-45, Rb-46, Rb-47, Rb-48	Present	51
2	Rb-5, Rb-6, Rb-16, Rb-21, Rb-22, Rb-24, Rb-43, Rb-54, Rb-55	Absent	09

Table 6: Number of days to produce microsclerotia in isolates of *R. bataticola* on potato dextrose agar

Sl. No.	Isolate	Number of days to produce microsclerotia (DAI)	Number of isolates
1	Rb-1, Rb-2, Rb-4, Rb-7, Rb-9, Rb-10, Rb-13, Rb-25, Rb-26, Rb-27, Rb-30, Rb-35, Rb-39, Rb-42, Rb-49, Rb-50, Rb-52, Rb-53, Rb-57	2-3	18
2	Rb-3, Rb-8, Rb-11, Rb-12, Rb-14, Rb-15, Rb-18, Rb-19, Rb-20, Rb-23, Rb-28, Rb-29, Rb-31, Rb-32, Rb-33, Rb-36, Rb-37, Rb-38, Rb-40, Rb-44, Rb-45, Rb-46, Rb-51, Rb-60, Rb-59, Rb-58	4-5	26
3	Rb-17, Rb-34, Rb-41, Rb-47, Rb-48, Rb-56	6 – 7	6

Table 7: Number of microsclerotia produced per microscopic field (10x) in isolates of *R. bataticola* on potato dextrose agar medium

Sl. No.	Isolate	No./microscopic field, (10x)	Number of isolates
1	Rb-2, Rb-19, Rb-20, Rb-23, Rb-26, Rb-31, Rb-32, Rb-33, Rb-34, Rb-36, Rb-40, Rb-41, Rb-42, Rb-46, Rb-47, Rb-48, Rb-52, Rb-56, Rb-58, Rb-59, Rb-60	0-25	21
2	Rb-1, Rb-3, Rb-4, Rb-7, Rb-8, Rb-9, Rb-10, Rb-11, Rb-12, Rb-14, Rb-15, Rb-17, Rb-18, Rb-25, Rb-27, Rb-30, Rb-35, Rb-37, Rb-38, Rb-39, Rb-44, Rb-45, Rb-49, Rb-50, Rb-51, Rb-57	26-50	26
3	Rb-13, Rb-28, Rb-29, Rb-53	51-100	4

Table 8: Shape of microsclerotia of isolates of *R. bataticola* under Q-capture image analyzer

Sl. No.	Isolate	Shape	Number of isolates
1	Rb-1, Rb-4, Rb-7, Rb-9, Rb-14, Rb-15, Rb-18, Rb-20, Rb-25, Rb-30, Rb-31, Rb-38, Rb-39, Rb-42, Rb-51, Rb-52, Rb-57, Rb-58, Rb-59, Rb-60	Round	20
2	Rb-10, Rb-11, Rb-12, Rb-17, Rb-26, Rb-27, Rb-29, Rb-32, Rb-36, Rb-37, Rb-40, Rb-48, Rb-46	Ovoid	13
3	Rb-2, Rb-3, Rb-8, Rb-13, Rb-19, Rb-23, Rb-28, Rb-33, Rb-34, Rb-35, Rb-41, Rb-44, Rb-45, Rb-47, Rb-49, Rb-50, Rb-53, Rb-56	Irregular	18

Table 9: Identity of *Rhizoctonia bataticola* isolates of chickpea sequencing and BLAST results

Sl. No.	Isolate	Identified as	Per cent homology	Ref. accession number
1.	Rb-1	*Rhizoctonia bataticola*	99%	HQ649832.1
2.	Rb-13	*Rhizoctonia bataticola*	99%	KX270355.1
3.	Rb-17	*Rhizoctonia bataticola*	99%	MG001962.1
4.	Rb-25	*Rhizoctonia bataticola*	99%	KX447538.1
5.	Rb-36	*Rhizoctonia bataticola*	99%	HQ392772.1
6.	Rb-44	*Rhizoctonia bataticola*	99%	HQ392794.1
7.	RRb-48	*Rhizoctonia bataticola*	99%	HQ392778.1
8.	Rb-56	*Rhizoctonia bataticola*	99%	KT862032.1

Table 10: Similarity coefficients of *R. bataticola* isolates based on RAPD analysis

	Rb-1	Rb- 2	Rb-3	Rb-4	Rb-5	Rb-6	Rb-7	Rb-8	Rb-9	Rb-10	Rb-11	Rb-12	Rb-13	Rb-14	Rb-15	Rb-16	Rb-17	Rb-18	Rb-19	Rb-20
Rb-1	1.0																			
Rb-2	0.92	1.0																		
Rb-3	0.65	0.40	1.0																	
Rb-4	0.54	0.70	0.62	1.0																
Rb-5	0.61	0.68	0.38	0.70	1.0															
Rb-6	0.47	0.67	0.57	0.46	0.45	1.0														
Rb-7	0.62	0.60	0.49	0.49	0.56	0.48	1.0													
Rb-8	0.65	0.68	0.36	0.57	0.70	0.43	0.53	1.0												
Rb-9	0.68	0.58	0.46	0.46	0.67	0.53	0.34	0.43	1.0											
Rb-10	0.47	0.45	0.63	0.66	0.55	0.53	0.34	0.50	0.36	1.0										
Rb-11	0.57	0.54	0.39	0.55	0.76	0.19	0.35	0.45	0.65	0.38	1.0									
Rb-12	0.50	0.34	0.29	0.29	0.35	0.42	0.5	0.43	0.33	0.5	0.14	1.0								
Rb-13	0.55	0.41	0.23	0.42	0.54	0.37	0.45	0.41	0.44	0.29	0.32	0.38	1.0							
Rb-14	0.55	0.33	0.47	0.41	0.42	0.18	0.49	0.76	0.46	0.46	0.39	0.42	0.50	1.0						
Rb-15	0.54	0.45	0.72	0.60	0.61	0.53	0.48	0.50	0.36	0.61	0.65	0.48	0.49	0.46	1.0					
Rb-16	0.73	0.46	0.70	0.65	0.54	0.52	0.40	0.57	0.52	0.76	0.37	0.54	0.14	0.61	0.60	1.0				
Rb-17	0.40	0.57	0.45	0.55	0.48	0.44	0.57	0.18	0.44	0.78	0.75	0.62	0.61	0.62	0.36	0.43	1.0			
Rb-18	0.47	0.39	0.54	0.40	0.71	0.70	0.45	0.43	0.53	0.45	0.38	0.41	0.71	0.72	0.53	0.36	0.52	1.0		
Rb-19	0.53	0.52	0.36	0.45	0.42	0.44	0.82	0.64	0.34	0.60	0.18	0.55	0.54	0.45	0.36	0.50	0.71	0.7	1.0	
Rb-20	0.60	0.63	0.53	0.39	0.54	0.68	0.61	0.47	0.44	0.36	0.37	0.61	0.48	0.61	0.52	0.50	0.50	0.60	0.58	1.0

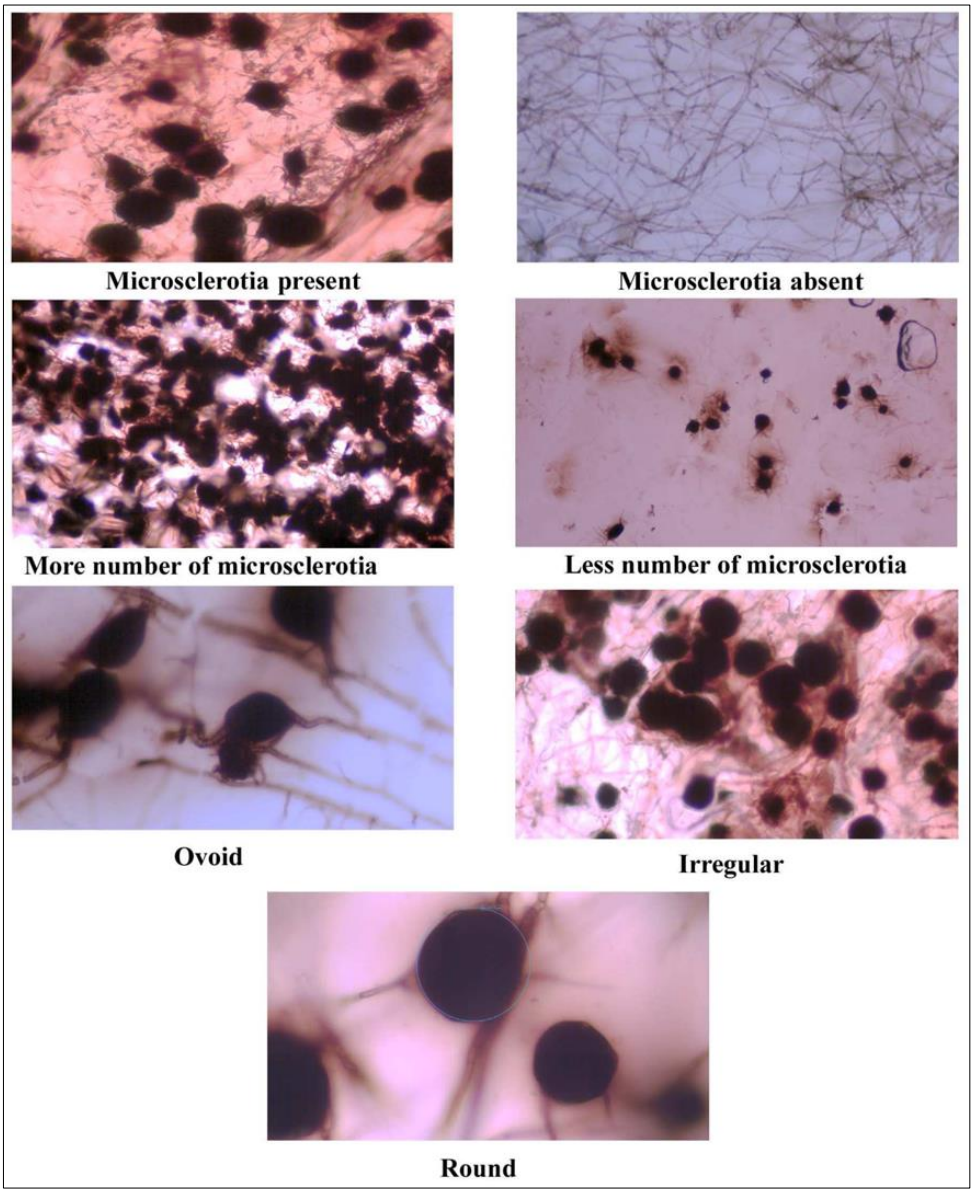

Fig 1: Diversity in Microsclerotial production in *R. bataticola* isolates

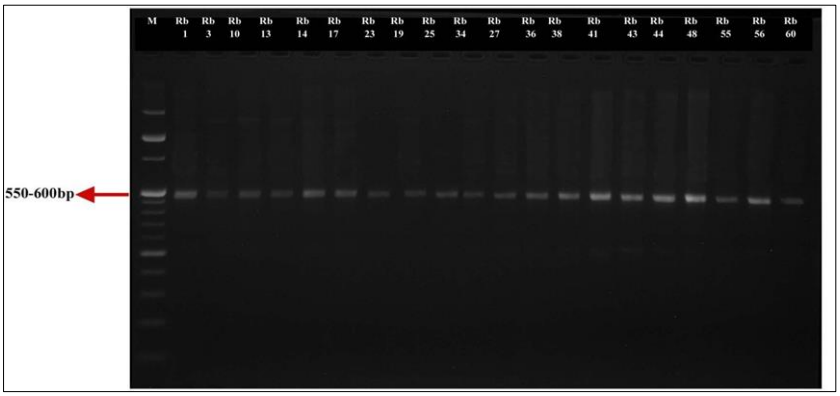

Fig 2: Amplified products of *R. bataticola* isolates using ITS1 and ITS4 primers

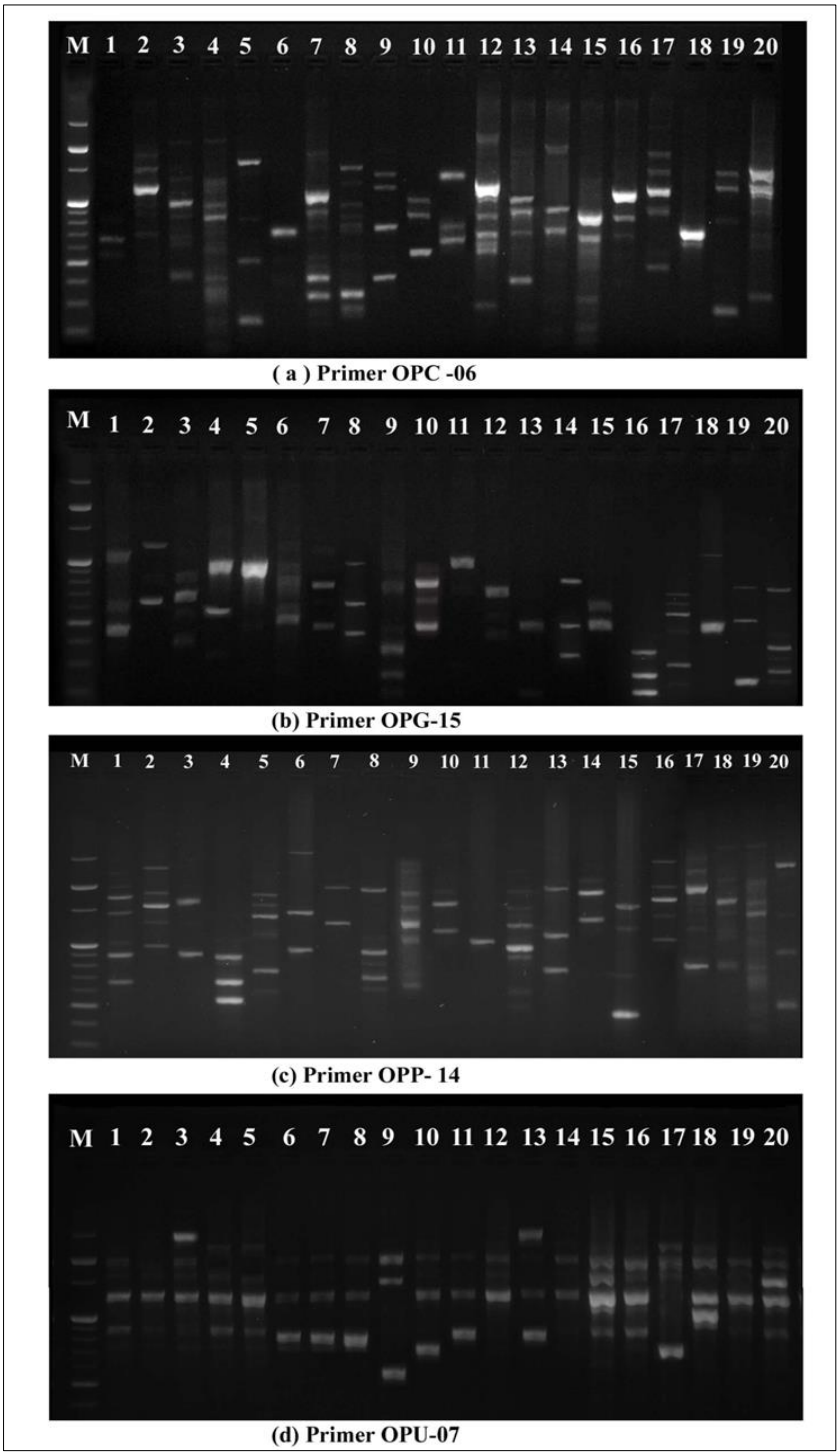

Fig 3: DNA profiling of *R. bataticola* isolates banding pattern with RAPD primers

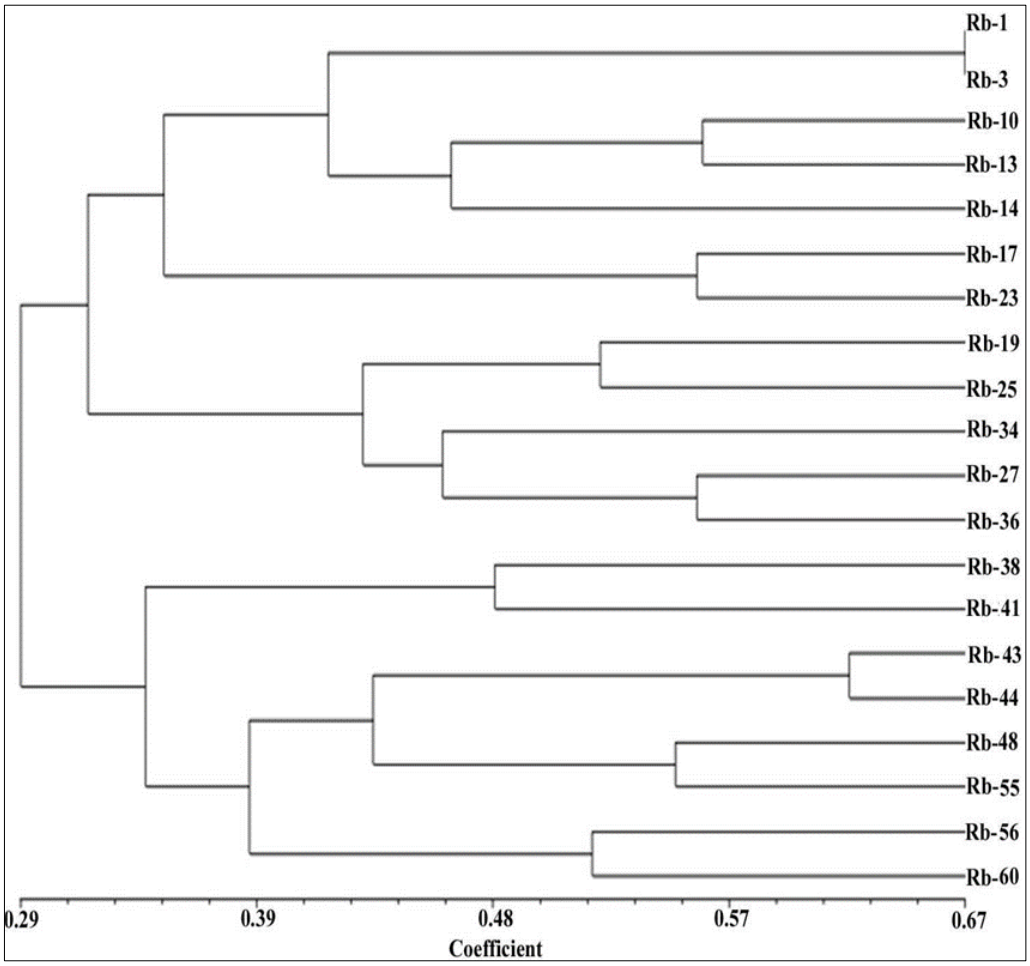

Fig 4: Hierachical horizontal dendrogram showing clustering of *R. batatico*

Results and Discussion

Isolation and identification of *R. bataticola*: The pathogen produced black, brown to grey coloured mycelium that become darker with age. The young hyphae were thin, hyaline, septate and dichotomously branched and later produce typical black sclerotia.

Cultural diversity of *R. bataticola* isolates

Radial growth: Radial growth of *R. bataticola* ranged from 23.90-90 mm (Table 1). Fifty-five isolates were fast growing (75-90 mm), two isolates moderate (60-75mm) and only three isolates slow growing (45-60 mm).

Colony morphology: Seventeen isolates recorded appressed growth, 13 velvety and rest of the 30 isolates showed fluffy growth pattern (Table 2).

Colony pigmentation: Black with grey aerial mycelial colony was found in 15 isolates, black pigmentation (15 isolates), grey (25 isolates) and white pista coloured (5 isolates) (Table 3).

Aerial mycelium: Twenty-eight isolates showed the production of arial mycelia and in 32 isolates aerial mycelia was absent (Table 4). Aghakhani and Dubey, (2009) [1] studied the variability of twenty-three isolates of *R. bataticola* causing dry root rot of chickpea collected from 10 different major chickpea growing states of India and reported that all isolates were highly variable in their cultural characters. Aghakhani and Dubey, (2009) [1] studied the variability of twenty-three isolates of *R. bataticola* causing dry root rot of chickpea collected from 10 different major chickpea growing states of India and reported that all isolates were highly variable in their cultural characters. Similarly, Manjunatha and Naik (2011) [5] reported that out of thirty isolates of *R. bataticola* collected from three major chickpea growing regions twelve isolates were fast in growth, another twelve were moderate and the remaining six isolates were slow in growth. Further, variability among the eleven isolates of *R. bataticola* from different pulse crops (redgram, greengram, cowpea, soybean, blackgram) isolates were categorized into linear, fluffy and linear at the end with fluffy growth at the centre (Sundravadana *et al*., 2012) [13].

Morphological diversity of *R. bataticola*

Microsclerotial production: Fifty-one isolates produced microsclerotia, while 9 did not produce microsclerotia (Table 5 and Fig. 1).

Number of days to produce microsclerotia: Eighteen isolates produced within 2-3 days, 26 within 4-5 days and 6 isolates 6-7 days (Table 6).

Number of microsclerotia produced: Twenty-one isolates produced sclerotial bodies in the range of 1-25 per microscopic field (10x), twenty-six (26-50 range) and four isolates (51-100 range) (Table 7 and Fig. 1).

Shape of the microsclerotia: Round shaped microsclerotia were observed in twenty isolates, ovoid (13) and irregular

(18) (Table 8 and Fig. 1). Similar results with respect to diversity in colony pigmentation of *R. bataticola* were observed by Manjunatha and Naik (2011) [5] who categorized isolates into three groups based on colony colour. Similarly, Purkayastha *et al*. (2004) [7] reported that sixteen isolates produced sclerotia in one day, thirteen isolates in three days and the rest of isolates taken five days in clusterbean out of 55 isolates.

Molecular characterization and Molecular diversity of *R. bataticola* isolates

Molecular characterization of *R. bataticola*: PCR amplification of ITS region of 18SrRNA with ITS primers produced amplified products size of 500-650 bp in all the twenty isolates (Fig. 2) indicating that all the isolates belong to genus *R. bataticola.* Further, sequences of eight selected isolates were obtained and BLAST data revealed that all eight isolates matched with the reference species of NCBI with 99% similarity and identified as *Rhizoctonia bataticola* (Table 9).

Genetic diversity in *R. bataticola* isolates: Four random primers *viz.,* OPC-06, OPG-15, OPP-14 and OPU-07 generated reproducible polymorphism in all twenty isolates and amplified products showed polymorphic and distinguishable banding pattern indicating the genetic diversity. A total of 389 reproducible and scorable polymorphic bands ranging approximately as low as 150 bp to as high as 1000 bp were generated (Fig. 3).

In dendrogram, all the twenty isolates grouped into two main clusters indicating there is genetic diversity in the isolates of *R. bataticola.* Cluster I contained twelve isolates, main cluster divided into two sub clusters, sub cluster I had seven isolates (Rb-1, Rb-3, Rb-14, Rb-27, Rb-25, Rb-60 and Rb-41) and sub cluster II had five isolates (Rb-36, Rb-48, Rb-10, Rb-13 and Rb-34). Main cluster II has two sub clusters, sub cluster I consisted of two isolates (Rb-55 and Rb-43) and in sub cluster II six isolates (Rb-19, Rb-23, Rb-17, Rb-38, Rb-44 and Rb-56) were grouped (Fig. 4). The similarity coefficient ranged from 14 to 92 per cent (Table 10). The maximum genetic similarity of 92 was between Rb-1 and Rb-3 whereas least genetic similarity (14 per cent) was between Rb-17 and Rb-27.

The results of present investigation are in confirmation with the studies conducted by Shekhar *et al*. (2006) [12] where they analyzed seven isolates of *R. bataticola*, incitant of maize rot through RAPD marker for genetic diversity. They observed that the most closely related isolates were Hyderabad and Delhi with an affinity percentage of 75.5 followed by Udaipur and Bangalore isolates with 62.9 per cent similarity. Pancheswar *et al*. (2012) [6] studied the genomic DNA of 21 isolates of R. *bataticola* of soybean from Jabalpur with 8 randomly selected decamer primers which amplified 64 RAPD marker loci. Out of these 64 bands, 29 bands (45.3%) were polymorphic.

Further, the phylogenetic tree based on rDNA-ITS analysis showed maximum number *R. bataticola* isolates were very diverse and did not depend on geographical origin. Both pathological and molecular data correlated with each other supported that the

R. bataticola present in India were very diverse and independent to their origin. Similarity coefficient values for 21 accessions of *R. bataticola* in RAPD marker was 0.69–0.98.

Conclusion

There was more diversity in *R. bataticola* isolates with respect to cultural and morphological characters. Universal ITS primers successfully amplified the DNA of all twenty isolates with amlicon size of 550- 600 bp indicating isolates belonged *to R. bataticola*. Further, nucleotide sequencing was done for ITS region of 18S rRNA in eight isolates representing different geographic regions and accession numbers were deposited in NCBI GeneBank, Maryland, USA. Four random RAPD generated reproducible polymorphism and amplified products with all the primers showed polymorphic and distinguishable banding pattern indicating the genetic diversity among twenty representative isolates of *R. bataticola*.

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